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cDNA Synthesis using the Universal RiboClone® System

By Sandra DeMars and Gary Kobs
Promega Corporation

Promega's Universal RiboClone® cDNA Synthesis System provides a convenient method for cDNA synthesis and cloning. In this update, we describe features of the reconfigured system and present representative data obtained from cDNA synthesis reactions using mRNA isolated from mouse liver.

Introduction

The construction of a representative cDNA library is one of the more challenging processes in molecular biology. A complex series of enzymatic steps is involved in copying mRNA into double-stranded cDNA and subsequently preparing the termini for vector ligation. cDNA libraries may be used in a variety of applications (1), including gene structure analysis, protein expression studies and DNA mapping studies.

Promega recently combined its range of products for cDNA synthesis and cloning into one versatile system, the Universal RiboClone® cDNA Synthesis System (Cat.# C4360). Like the earlier RiboClone® cDNA Synthesis Systems, the Universal RiboClone® cDNA Synthesis System contains all the reagents needed to synthesize double-stranded cDNA from up to 40µg of mRNA. Additionally, the system includes enzymes and reagents to prepare the cDNA for cloning into a suitable vector.

The Universal RiboClone® cDNA Synthesis System is based on the method described by Okayama and Berg (2), with modifications by Gubler and Hoffman (3). First strand synthesis is driven by AMV (Avian Myeloblastosis Virus) Reverse Transcriptase and either Random Hexameric Primers or an Oligo(dT) Primer, followed directly by second strand replacement synthesis using RNase H and DNA Polymerase I. After treatment with T4 DNA Polymerase to flush the ends, the double-stranded cDNA molecules are prepared for cloning by the ligation of *Eco*R I Adaptors. Excess adaptors are removed by chromatography using Sephacryl® Matrix and Spin Columns. The system also includes a Positive Control RNA which may be used to assess the efficiency of cDNA synthesis and ligation reactions.

Choice of primers

The classical method of cDNA synthesis uses oligo(dT) to prime first strand synthesis of cDNA from poly(A)$^+$ RNA templates. Random hexameric primers provide an alternative procedure by which first strand cDNA synthesis is initiated from internal sites within the mRNA molecule (4), and are useful for priming cDNA synthesis from mRNA molecules that are not polyadenylated or from RNA isolated from prokaryotic sources. The Universal RiboClone® cDNA Synthesis System includes Oligo(dT) and Random Hexameric Primers to provide a choice of priming methods. Both types of primers yield cDNA products that are cloned in a random orientation.

For directional cDNA cloning, an oligo(dT) containing primer-adaptor may be used instead of the primers included in the system. *Not* I (Cat.# C1591) and *Xba* I (Cat.# C1011) Primer-Adaptors can be purchased separately for use with any compatible vector. The *Not* I Primer-Adaptor is included with Promega's Lambda gt11 *Sfi-Not* Vector (Cat.# T3220) and an *Xba* I Primer-Adaptor is included with the LambdaGEM®-2 and LambdaGEM®-4 Vectors (Cat.# T3270 and T3290, respectively).

Orientation-specific or directional cDNA cloning is valuable in some applications. In expression vectors (e.g., Lambda gt11 *Sfi-Not*), this method of cDNA cloning can increase by a factor of two the probability of expressing the insert as the correct polypeptide. For *in vitro* transcription vectors, such as the LambdaGEM®-2 and LambdaGEM®-4 Vectors, all inserts are cloned in the same orientation relative to the promoters for T7 and SP6 RNA Polymerase. Total DNA from the resulting library can be transcribed to obtain either sense or antisense RNA probes, respectively, that represent all of the sequences in the library.

cDNA synthesis from mouse liver mRNA

Optimal conditions for first strand synthesis must be established to achieve maximal yield of full-length cDNA. This is especially important for rare or long messenger RNAs. RNA with significant secondary structure, such as GC-rich areas or hairpin structures, may also be difficult to clone due to the inability of reverse transcriptases to read through these regions. This problem can be alleviated by using higher temperatures during first strand synthesis. The ability of AMV Reverse Transcriptase to function at temperatures higher than 42°C makes it the enzyme of choice for reverse transcription of problematic mRNAs (5-7).

We compared the size distribution of cDNA products obtained using two different temperatures for first strand synthesis. We first isolated mRNA from mouse liver using the PolyATtract® System 1000 (Cat.# Z5420) and used this mRNA as a template for first strand cDNA synthesis using the Oligo(dT) Primer supplied with the Universal RiboClone® System. The conditions used were essentially as described in the Technical Manual (8) except that reactions were performed at 42°C and 48°C. Tracer reactions were performed on an aliquot of the first strand reaction mixture using [^{32}P]dCTP, for quantitative and qualitative analysis of the products. The unlabeled first strand reaction products were then carried over into second strand synthesis and second strand tracer reactions were performed. Each first and second strand tracer reaction was analyzed by electrophoresis in a 1.4% alkaline agarose gel (9).

The results showed that raising the first strand reaction temperature to 48°C had no effect on the size distribution of first and second strand reaction products (Figure 1). The yield of first strand reaction products was equivalent at both temperatures, as determined by trichloroacetic acid (TCA) incorporation assays (data not shown). Therefore, AMV Reverse Transcriptase may prove useful in resolving problems with cDNA synthesis caused by mRNA secondary structure.

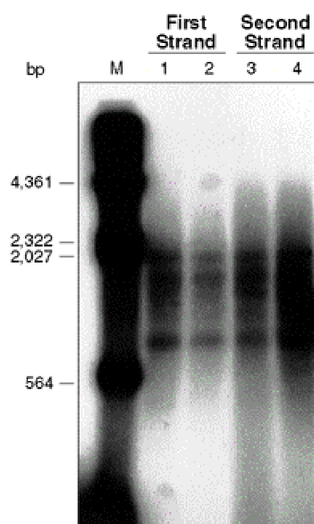

Figure 1. Analysis of cDNA synthesized using the Universal RiboClone® System. cDNA was synthesized from mouse liver poly(A)$^+$ RNA using the Oligo(dT) Primer and AMV Reverse Transcriptase at 42°C and 48°C. Tracer reactions were performed on a portion on the first strand reaction mixtures using [^{32}P]dCTP. After the completion of the first strand reaction, the unlabeled reaction products were used for second strand synthesis and tracer reactions. The first and second strand tracer reactions were analyzed in a 1.4% alkaline agarose gel (9). Lanes 1 and 2, first strand tracer reactions performed at 42°C and 48°C, respectively; Lanes 3 and 4, second strand tracer reactions from first strand reactions prepared at 42°C and 48°C, respectively; Lane M, Promega's Lambda DNA *Hin*d III Marker (Cat.# G1711) end-labeled with [gamma-^{32}P]ATP (sizes of representative bands are indicated in bp).

Summary

The Universal RiboClone® cDNA Synthesis System provides all the necessary reagents for cDNA synthesis from up to 40µg of mRNA, and preparation of the products for random orientation cloning into a suitable vector. The standard first strand synthesis reaction is carried out at 42°C; however, we have found that raising the reaction temperature to 48°C does not adversely affect the size distribution and yield of cDNA synthesized with the Oligo(dT) Primer.

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Ordering Information

Product	**Cat.#**
Universal RiboClone® cDNA Synthesis System	C4360

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